

CLAIMS

1. A method of detecting *Mycobacterium* species present in a biological sample, comprising the steps of:
 - providing a biological sample containing nucleic acid from at least one *Mycobacterium* species comprising a *Mycobacterium* 16S ribosomal RNA (rRNA) or DNA encoding 16S rRNA;
 - amplifying the *Mycobacterium* 16S rRNA or DNA in an *in vitro* nucleic acid amplification mixture comprising at least one polymerase activity, and at least two primers having sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO: 34, SEQ ID NO:37 and SEQ ID NO:38 to produce amplified *Mycobacterium* nucleic acid; and
 - detecting the amplified *Mycobacterium* nucleic acid by detecting a label associated with the amplified *Mycobacterium* nucleic acid.
2. The method of Claim 1, further comprising in the steps of:
 - adding to the biological sample at least one capture oligonucleotide that specifically hybridizes to the *Mycobacterium* 16S rRNA and an immobilized nucleic acid that hybridizes to the capture oligonucleotide under hybridizing conditions to produce a hybridization complex; and
 - separating the hybridization complex from other components of the biological sample before the amplifying step.
3. The method of Claim 1, wherein the amplifying step amplifies 16S rRNA or DNA encoding 16S rRNA from *M. tuberculosis* or a *Mycobacterium* other than *tuberculosis* (MOTT) species.
4. The method of Claim 1, wherein the amplifying step amplifies 16S rRNA or DNA encoding 16S rRNA from *M. abscessus*, *M. africanum*, *M. asiaticum*, *M. avium*, *M. bovis*, *M. celatum*, *M. chelonae*, *M. flavescens*, *M. fortuitum*, *M. gastri*, *M. gordonae*, *M. haemophilum*, *M. intracellulare*, *M. interjectum*, *M. intermedium*, *M. kansasii*, *M. malmoense*, *M. marinum*, *M. non-chromogenicum*, *M. paratuberculosis*, *M. phlei*, *M. scrofulaceum*, *M. shimodei*, *M. simiae*, *M. smegmatis*, *M. szulgai*, *M. terrae*, *M. triviale*, *M. tuberculosis*, *M. ulcerans* or *M. xenopi*.
5. The method of Claim 1, wherein the detecting step uses at least one probe that hybridizes specifically to the amplified *Mycobacterium* nucleic acid.
6. The method of Claim 5, wherein the detecting step uses at least one labeled probe that hybridizes specifically to the amplified *Mycobacterium* nucleic acid.
7. The method of Claim 5, wherein the detecting step uses a plurality of probes that hybridize specifically to the amplified *Mycobacterium* nucleic acid.

8. The method of Claim 1, wherein the amplifying step uses a combination of at least a first primer and a second primer, wherein the first primer is selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:12, and the second primer is selected from the group consisting of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 and SEQ ID NO:38.

5 9. The method of Claim 8, wherein the amplifying step uses a combination of at least a first primer and a second primer, wherein the first primer is selected from the group consisting of SEQ ID NO:7 to SEQ ID NO:12, and the second primer is selected from the group consisting of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 and SEQ ID NO:38.

10 10. The method of Claim 8, wherein the amplifying step uses a combination of at least a first primer and a second primer selected from the group consisting of:

the first primer having the sequence of SEQ ID NO:7, and the second primer having the sequence of SEQ ID NO:13;

the first primer having the sequence of SEQ ID NO:7, and the second primer having the sequence of SEQ ID NO:14;

15 the first primer having the sequence of SEQ ID NO:7, and the second primer having the sequence of SEQ ID NO:15;

the first primer having the sequence of SEQ ID NO:7, and the second primer having the sequence of SEQ ID NO:16;

20 the first primer having the sequence of SEQ ID NO:8, and the second primer having the sequence of SEQ ID NO:13;

the first primer having the sequence of SEQ ID NO:8, and the second primer having the sequence of SEQ ID NO:14;

the first primer having the sequence of SEQ ID NO:8, and the second primer having the sequence of SEQ ID NO:15;

25 the first primer having the sequence of SEQ ID NO:9, and the second primer having the sequence of SEQ ID NO:13;

the first primer having the sequence of SEQ ID NO:9, and the second primer having the sequence of SEQ ID NO:14;

30 the first primer having the sequence of SEQ ID NO:9, and the second primer having the sequence of SEQ ID NO:15;

the first primer having the sequence of SEQ ID NO:10, and the second primer having the sequence of SEQ ID NO:16;

the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:13;

the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:16;

5 the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:17;

the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:18;

10 the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:19;

the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:20;and

the first primer having the sequence of SEQ ID NO:12, and the second primer having the sequence of SEQ ID NO:15.

15 11. The method of Claim 8, wherein the amplifying step uses a combination of the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:16, SEQ ID NO:30 or SEQ ID NO:37.

12. The method of Claim 8, wherein the amplifying step uses a combination of the first primer having the sequence of SEQ ID NO:11, and two second primers having the sequences SEQ ID 20 NO:16 and SEQ ID NO:37.

13. A composition for amplifying in an *in vitro* amplification reaction a *Mycobacterium* 16S rRNA sequence or a DNA encoding 16S rRNA, comprising one or more oligonucleotides having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO: 34, SEQ ID NO:37 and SEQ ID NO:38.

25 14. The composition of Claim 13, wherein the composition comprises:

at least one first oligonucleotide having the sequence of any one of SEQ ID NO:1 to SEQ ID NO:12, and

at least one second oligonucleotide having the sequence of any one of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 or SEQ ID NO:38.

30 15. The composition of Claim 14, wherein the composition comprises:

at least one first oligonucleotide containing the sequence of any one of SEQ ID NO:7 to SEQ ID NO:12, and

at least one second oligonucleotide containing the sequence of any one of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 or SEQ ID NO:38.

16. A kit containing any or more oligonucleotides having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:34, SEQ ID NO:37 and SEQ ID NO:38.
- 5 17. The kit of claim 16, containing
at least one first oligonucleotide having the sequence of any one of SEQ ID NO:1 to SEQ ID NO:12, and
at least one second oligonucleotide having the sequence of any one of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 or SEQ ID NO:38.
- 10 18. The kit of claim 17, containing
at least one first oligonucleotide containing the sequence of any one of SEQ ID NO:7 to SEQ ID NO:12, and
at least one second oligonucleotide containing the sequence of any one of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 or SEQ ID NO:38.